



## Screening of antioxidant potential of *Citrullus Colocynthis* methanolic extract

Sudhanshu<sup>1</sup>, Nidhi Rao<sup>1</sup> Sandhya Mittal<sup>1</sup> and Ekta Menghani\*<sup>2</sup>

<sup>1</sup>Suresh Gyan Vihar University, Jaipur

<sup>2</sup>Mahatma Gandhi Institute of Applied Sciences, JECRC Campus, Jaipur-22. India

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### ABSTRACT

Medicinal plants are the spine of long-established systems of medication all the way through the world. They are worthless, barely credible and conventional source for the remedial assortment of diseases in the appearance of medicines. The purpose of our current study was to estimate the antioxidant property of *Citrullus colocynthis* methanolic extract. *Citrullus colocynthis* belongs to family Cucurbitaceae, is a therapeutic plant conventionally used as an abortifacient and to indulge constipation, oedema, bacterial infections, cancer and diabetes. The enthusiasm in the rear of this study was to emphasize the consequence of conventionally consumed plants as a persuasive resource of bioactive compounds. Phytochemical Screening of methanolic extract showed the occurrence of a variety of phytoconstituents that are accountable for pharmacological activities. The Antioxidant activity of methanolic extract was study spectrophotometrically by 1,1-diphenyl-2-picryl hydrazyl as well as free radical scavenging method. The results of present study revealed that the methanolic extract of *Citrullus colocynthis* possesses considerable antioxidant activity due to its free radical scavenging property.

**Keywords:** *Citrullus colocynthis*, Phytochemical Screening, Antioxidant Activity, 1,1-diphenyl-2-picryl hydrazyl, Free radicals.

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### INTRODUCTION

The exploit of Natural medication is a incredibly affluent folklore predominantly amongst the people of India, China, Egypt and Brazil. Conventional remedial System in India includes Ayurveda, Siddha and Unani which are based on make the most of of innate remedy [1]. Natural Medicines encompass since an assortment of sources resembling terrestrial plants, terrestrial microorganism, marine organisms, terrestrial vertebrates and invertebrates [2]. Although medicines obtained as of plant sources are extremely valuable. Natural medicine are in addition acknowledged as Botanical medicine or phytomedicine which refers to employ seeds, berries, roots, leaves, bark and flowers for curative purposes [3].

Readily available an interest is increasing wide-reaching on restorative values of natural products. The environment provides the mankind enormous salutary flora with a extensive diversity of medicinal potential. The revitalization of significance in plant imitative drugs is principally due to in progress prevalent conviction that "green medicine" is not dangerous and additional steady than the valuable synthetic drugs lots of of which have undesirable side effects and also necessitate is to screen a numeral of remedial vegetation for talented biological activity [4]. In topical years, phytochemicals in vegetables include a immense pact of consideration generally on their role in preventing diseases caused as a consequence of oxidative stress which release reactive oxygen species such as singlet oxygen in addition to a variety of radicals as a destructive side outcome of aerobic metabolism. These radicals are probably implicated in a number of disorders together with cardiovascular malfunctions, tissue injury, DNA damage and tumor promotion. Numerous studies put forward that antioxidants might prevent accumulation of these reactive oxygen species along with beneficial for management of these pathologies. Diets prosperous in fruits also vegetables has been associated constantly with condensed menace of a variety of tumors, particularly epithelial cancers of

respiratory and gastrointestinal tract. The phrase antioxidant refers to the commotion of frequent vitamins, minerals and other phytochemicals to defend adjacent to the damage cause by reactive oxygen species [5]. Free radicals are concerned in quite a lot of degenerative diseases like as atherosclerosis, diabetes, arthritis and cancer [6]. Artificial antioxidants be capable of be carcinogenic so innate antioxidants can establish a promising unconventional. Medicinal plants acquire a assortment of compounds of acknowledged therapeutic property [7,8,9]. Based on the narrative review and erstwhile imperative uses of Cucurbitaceae family, this family is frequently known for melons, gourds or cucurbits plus include crops like cucumbers, squashes (including pumpkins) in addition to luffas and convenient are about 118 genera in this family which includes 825 species [10]. Fruits along with Peel part of this family has been mainly explored for their pharmacological activities moreover it has been well established by ayurveda and traditional system of medicine [11].

*Citrullus colocynthis* is a remedial plant belongs to the family Cucurbitaceae. It is a broad extend annual unsophisticated plant, procumbent herb having diminutive flowers amid of yellow colour. The fruit is extremely bitter. It grows speedy in the grimy soils as well as prevalent in diverse parts of Saudi Arabia. This lodge is used as anticancer agent in numerous drugs and also used as antipyometra in animals [12]. It has been used in herbal treatment of diabetes, edema, bacterial infection as well as cancer. So the present study was designed to evaluate the antioxidant and phytochemical activity of *Citrullus colocynthis*.

## EXPERIMENTAL SECTION

### Collection:

Authentic samples: Various market samples of *Citrullus colocynthis* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

### Identification:

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGias, Jaipur (Rajasthan).

### Processing of plant materials:

During the course of the study each sample was screened for its foreign matter and milled, before use.

### Experimental details:

Present studies were performed on *Citrullus colocynthis* for the following studies-

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

## 1. PHYTOCHEMICAL SCREENING

Phytochemical screening was performed using standard procedure:

### TEST FOR REDUCING SUGARS (FEHLINGS TEST)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

### TEST FOR TERPENOIDES (SALKOWSKI TEST)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

### TEST FOR FLAVONOIDES

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

### TEST FOR TANNINS

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

**TEST FOR SAPONINS**

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**TEST FOR ALKALOIDS**

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

**2. ANTIOXIDANT ACTIVITY****Preparation of test extracts**

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

**Preparation of DPPH**

DPPH (2, 2'-diphenyl-1-picrylhydrazyl,  $C_{18}H_{12}N_5O_6$ ; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

**Qualitative assay**

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25  $\mu$ l was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

**Quantitative assay**

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations ( $10^2 \mu$ g to  $10^{-3} \mu$ g/ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance ( $RC_{50}$ ) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

**RESULTS AND DISCUSSION**

**Table 1: Showing Optical density of *Citrullus colocynthis* on different concentrations.**

CONCENTRATION ( $\mu$ g/ml)	O.D (nm)
0.001	1.720
0.01	1.679
0.1	1.608
1	1.603
10	1.448
100	1.174
1000	1.122

In the present investigation it was showed that the maximum optical density comes out to be 1.720 nm which is at the concentration  $10^{-3} \mu$ g/ml and the smallest optical density is 1.122 nm which is at the concentration  $10^3 \mu$ g/ml where as the other shows comparable O.D at different concentrations i.e. 1.679 nm at  $10^{-2} \mu$ g/ml, 1.608 nm at  $10^{-1} \mu$ g/ml, 1.603 nm at 1  $\mu$ g/ml, 1.448 nm at  $10^1 \mu$ g/ml, 1.174 nm at  $10^2 \mu$ g/ml.

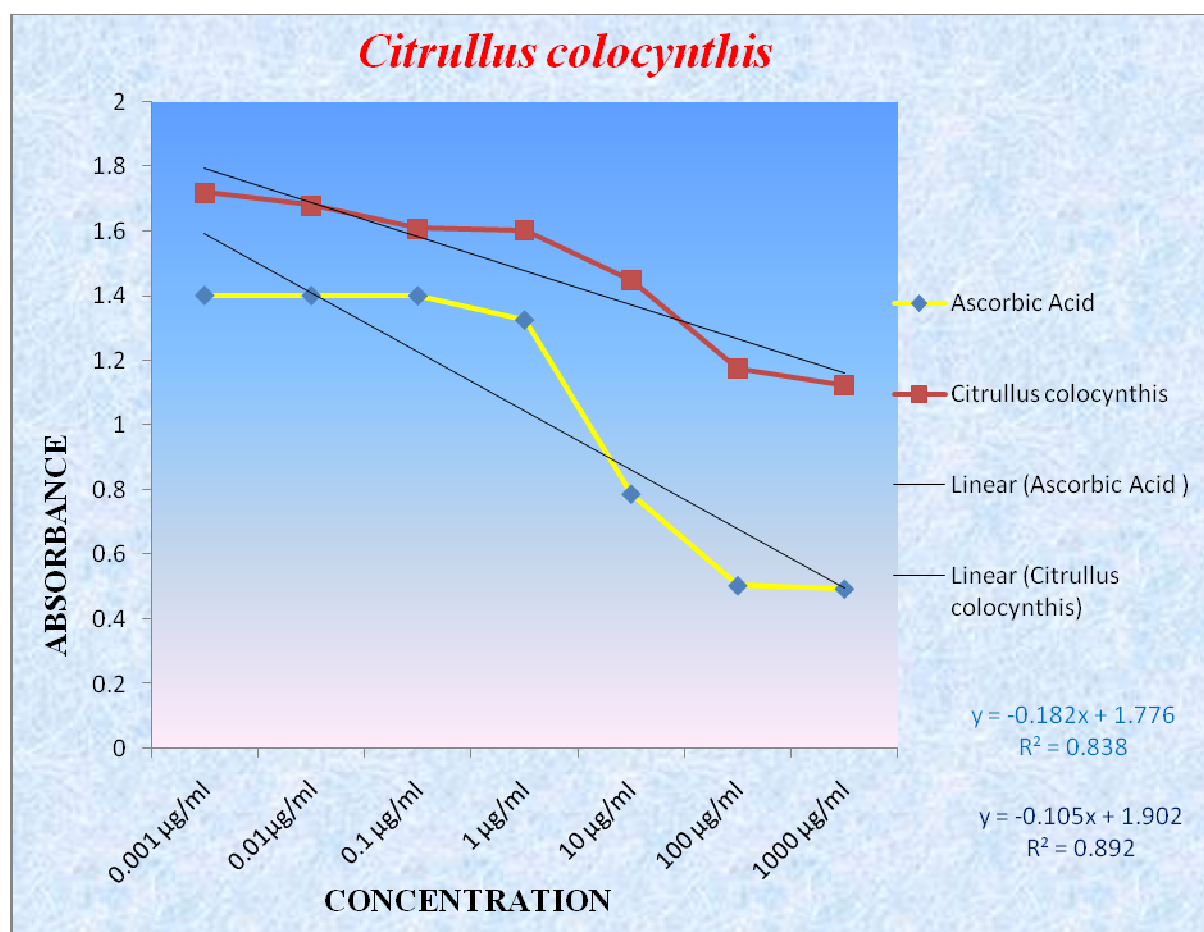


Fig 1: Graph showing Antioxidant Activity of *Citrullus colocynthis* at different concentration.

The DPPH radical scavenging activity of *Citrullus colocynthis* is shown in the graph in which the present investigations of antioxidant activity of *Citrullus colocynthis* showed appreciable activity against the DPPH assay method where the regression line clear cut showed the effectiveness of it as it's have potentials which are comparable to ascorbic acid. The antioxidant activity of *Citrullus colocynthis* in methanolic extract using DPPH assay method shows appreciable activity comparable to standard ascorbic acid. The straight line showed  $Y = -0.182x + 1.776$  & regression = 0.838 whereas, in above drug the straight line is  $Y = -0.105x + 1.902$  & regression = 0.892.

Table 2: Showing phytochemical screening results of *Citrullus colocynthis*

<i>Citrullus colocynthis</i>						
TEST	Reducing Sugar	Saponin	Tannin	Terpenoides	Flavonoides	Alkaloides
	-	-ve	-ve	-	+	+

The phytochemical screening of *Citrullus colocynthis* shows the occurrence of alkaloids, and flavonoids whereas it shows the absence of tannin and saponin respectively. The screening of the *Citrullus colocynthis* shows only a minute quantity of differences in the module of the hardnosed plants. The incidence of quercetin in enormous quantity is logically proportional to the antioxidant activity so it is manifestly shows that happening of flavonoids will demonstrate the antioxidant activity as well as responsible for the free radical scavenging effects observed.

## CONCLUSION

In current years, conservation and exploit of medicinal plants has taken generous amount of awareness. It has been used wide-reaching by the confined and subsidiary communities for curing a variety of diseases from primeval times. Generally of the plant species are in addition used as foodstuff enhancement all along with its oral decoctions. The present research is an endeavour in doing so. Since the present investigation it is over and done with the methanolic extract of *Citrullus colocynthis* showed antioxidant activity by free radical scavenging property in addition to it can be used as effective resource of natural antioxidant agent. The shrink in absorbance is unavailable as a appraisal of the extent of radical scavenging. Free radical scavenging capacity of the methanolic extracts as well

as standard (Ascorbic Acid), measured by DPPH assay was experiential. Moreover the Phytochemical screening of *Citrullus colocynthis* shows only some differences in which it possess a large amount of alkaloids and flavonoids. The prevalence of flavonoids in the plants is expected to be conscientious for the free radical scavenging effects experiential.

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